PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 9/20, 9/22, 9/26

A1

(11) International Publication Number: WO 90/0978

(43) International Publication Date: 7 September 1990 (07.09.9(

(21) International Application Number: PCT/US90/00900

(22) International Filing Date: 22 February 1990 (22.02.90)

(30) Priority data:
313,953 22 February 1989 (22.02.89) US

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(81) Designated States: AT (European patent), BE (Europea patent), CH (European patent), DE (European patent) DK (European patent), ES (European patent), FR (Eu ropean patent), GB (European patent), IT (Europea patent), JP, LU (European patent), NL (European pa tent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending th claims and to be republished in the event of the receipt o amendments.

(54) Title: DELIVERY SYSTEM FOR CONTROLLED RELEASE OF BIOACTIVE FACTORS

(57) Abstract

A composition and method for controlled release of water-soluble proteins comprising a surface-eroding polymer matrix and water-soluble bioactive factors is described. The composition bioerodes in the biological environment of the subject at a controlled rate, thereby releasing the water-soluble proteins at a rate which allows them to interact with local cell populations.

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DELIVERY SYSTEM FOR CONTROLLED RELEASE OF BIOACTIVE FACTORS

Background

The sustained administration of drugs over an extended period of time has significant medical and practical advantages in clinical practice. In recent years, much research has been done in developing systems for the sustained release of biologically active substances, particularly drugs, over periods of time. The purpose of these systems is to dispense the drug or other substance in a controlled manner at a selected physiological site. In the case of drugs used for therapy, presenting the drug in the most efficacious manner to effect treatment is desirable, while simultaneously minimizing complications which may occur as a result of the drug delivery.

Presently available systems for the sustained release of drugs are generally polymeric compositions where the drug or agent is either an integral part of the polymer matrix or layered or contained as a discrete portion of the device. For example, Folkman and Langer in U.S. Patent 4,291,797 describe a delivery device for macromolecules in which the macromolecule is interspersed throughout the polymer matrix.

In U.S. Patent 4,767,628, Hutchinson describes a delivery vehicle formed of polylactide polymer and an acid stable polypeptide interspersed in the matrix.

Higuchi in U.S. Patent 3,625,214 describes a sustained release drug delivery device according to a defined release profile by layering the drug and a bioerodible polymer.

Michaels in U.S. Patent 3,867,519 describes a sustained drug delivery device wherein release of the drug is controlled by the composition of an anionic polyvalent metal cation cross-linked polyelectrolyte.

In U.S. Patent 4,093,709 Choi and Heller describe a controlled release device formed from orthoester and orthocarbonate polymers.

While the above systems are useful, they are not appropriate for some applications, such as delivering water-soluble bioactive factors which react with a local cell population at a physiological site. A need exists for systems that can successfully deliver these agents which have favorable release kinetics and allow soluble agents to interact with local cells.

Summary of the Invention

The invention relates to a composition and method for the controlled administration of a bioactive substance to a local cell population in a subject. The composition comprises a bioerodible, surface-eroding polymer having the bioactive

substance interspersed throughout the matrix, which erodes in the biological environment, releasing the bioactive substance to the selected area. The invention also includes a method for delivering water-soluble local-acting substances, particularly proteins, to a specific site in the body of an animal.

The composition and method of the invention allows water-soluble proteins to interact with local cell populations. These proteins are soluble in the physiological environment, and are generally ineffective when introduced in vivo into a biological site because they quickly become diluted and dispersed in the body. The present composition releases soluble proteins directly to a selected site in a concentration sufficient to permit the proteins to interact with the local cell population. The surface-eroding polymers used in the present composition are biocompatible and bioerode in the physiological environment, allowing heterogeneous degradation from the surface of the device, which leads to near zero-order release kinetics. The degradation products of the surface-eroding polymers are non-mutagenic, non-cytotoxic and have a low teratogenic potential.

Detailed Description of the Invention

The present invention provides a system for the controlled or sustained release of bioactive substances which interact with local cell populations at a physiological site. The composition is formed

from a bioerodible, surface-eroding polymer and the bioactive substance. The "sustained" or "controlled" release of the substance may be either continuous or discontinuous.

The composition of the present invention comprises a bioerodible polymer matrix and a bioactive substance incorporated therein which, when placed in an aqueous physiological environment, releases the bioactive substance in a continuous manner until essentially all of the substance has been released, and the polymer has eroded away. The term "matrix" as used herein denotes a carrier, polymeric phase for the interspersed bioactive substance, which bioerodes in the environment of use, releasing the bioactive substance.

Bioerodible polymers suitable for use in the present invention are polymers which break down or disintegrate over a prolonged period of time when placed in contact with biological fluids. Surface-eroding bioerodible polymers are preferred for use in the present composition. Surface-eroding polymers are, generally, polymers having hydrophobic backbones and hydrolytic linkages, which bioerode from the surface at a constant rate in a biological environment. Surface eroding polymers include polyanhydrides and polyorthoesters.

Polyanhydride polymers are particularly useful for the present compositions. Polyanhydride polymers have several properties which are desirable in a biodegradable, polymeric controlled-release system. These polymers have a hydrophobic backbone

and a water labile linkage, which allows heterogeneous degradation in vivo from the surface of the polymer, leading to near zero-order release kinetics. The water-labile anhydride linkage provides the basis for the use of a variety of backbones, each having a unique degradation rate. Thus, the rate of degradation in vivo can be controlled by controlling the length and composition of the polymer backbone. Examples of polyanhydrides which can be used in the present invention include poly[bis(p-carboxyphenoxy) propane anhydride] (PCPP) and poly[bis(p-carboxy) methane anhydride] (PCPM). Co-polymers of polyanhydrides with other substances can also be used. For example, a co-polymer of PCPP and sebacic acid (PCPP-SA) has been shown to be an appropriate material to form a delivery device for soluble proteins. The degradation products of the polyanhydrides are non-mutagenic and non-toxic. In vivo toxicity studies have shown that polyanhydrides have excellent local system biocompatibility. The characteristics of polyanhydride drug carriers for systemic drugs is described by Leong et al. Leong et al., J. Biomed. Mat. Res., 19:941-955 (1985), Leong et al. J. Biomed. Mat. Res., 20:51-64 (1986).

Polyanhydride delivery vehicles of the present invention can be used to deliver highly soluble bioactive factors or substances which regulate local cellular events. These factors are generally characterized in that in vitro they produce a response in a cell population, but do not produce a response when used directly in vivo. These

substances or factors must be in the vicinity of the cells which they act upon to be effective. These factors are generally water-soluble polypeptides or proteins, which, when introduced into a physiological environment in vivo, are soluble in the environment, and so become too diluted to act locally. Bioactive factors having these characteristics include factors involved in wound healing or angiogenesis, such as TGF-beta, EGF, FGF and PDGF, which act upon local cell populations.

The term "water" as used herein (e.g., water-soluble), includes biological fluids, saline and physiologically acceptable buffer.

In one embodiment of the present invention, bioactive factors which promote chondrogenesis and osteogenesis are used. The protein(s) involved in chondrogenesis/osteogenesis interact with the local cells to influence their proliferation and cytodifferentiation. Transmembrane experiments have shown that the bioactive factor(s) responsible for the chondro-osteoinduction are soluble in body fluids. Nogami and Urist, Calcif. Tissue Res., 19:153-163 (1975); Urist et al., Arch. Surg., 112:612-619 (1977). It has been demonstrated that cold water-soluble proteins from bone matrix extracts will initiate chondrogenesis in in vitro assay systems. Syftestad and Caplan, Dev. Biol., 104:348-356 (1984); Syftestad et al., Differentiation, 29:230-237 (1985); Lucas et al., Differentiation, 37:47-52 (1987). However, due to their solubility, these protein fractions do not

induce osteogenesis in vivo when implanted alone. It has now been shown that when these proteins are properly formatted in a controlled-release vehicle, they are capable of inducing cartilage and bone in vivo.

Osteogenesis is initiated by an interaction between diffusable bone matrix-derived bioactive factor(s) and local ingrowing cell populations. Reddi and Huggins, Proc. Nat'l. Acad. Sci. USA, 69:1601-1605 (1972). These diffusable factors can be extracted from the demineralized matrix. However, these factors are very soluble in physiological solutions and will not initiate osteogenesis when implanted alone into an ectopic site. (Table 1) Demineralized bone matrix may be viewed as a "natural" sustained release vehicle which releases the soluble bioactive factor(s) in an effective manner. The present invention allows the dose and rate of release of bioactive factors exhibited by pieces of demineralized bone matrix to be mimicked.

The chondro/osteogenic water-soluble proteins used in the present embodiment of the invention are a complex mixture of proteins. Although considerable research has been done to isolate the chondro/osteogenic protein from bone matrix, to date no protein has been unequivocally purified to homogeneity. Urist et al., Proc. Nat'l. Acad. Sci. USA, 81:371-375 (1984); Seyedin et al., Proc. Nat'l. Acad. Sci. USA, 82:2267-2271 (1985); Sampath et al., Proc. Nat'l Acad. Sci. USA, 84:7109-7113 (1987); Sen

et al., Development and Diseases of Cartilage and Bone Matrix, pp. 201-219, A. Sen and T. Thornhill (eds.), Alan R. Liss, Inc., New York (1987); Wang et al., Proc. Nat'l. Acad. Sci. USA, 85:9484-9488 (1988). Ectopic endochondral ossification occurs through a cascade of events which include recruitment of the local responsive cells, proliferation of the cells, and cytodifferentiation. Each of these steps is thought to involve different bioactive factors, i.e., chemoattractants, mitogens, and chondro/osteogenic factor(s). Reddi, Current Adv. in Skeletogenesis, pp. 77-86, M. Silberman and H. Slavkin (eds.), Excerpta Medica, Amsterdam (1982). A crude mixture of proteins is more likely to contain all the necessary bioactive factors. The water-soluble proteins have been shown to be unable to induce ectopic osteogenesis when implanted alone, but to support the osteogenic cascade when incorporated into the herein described composition. When a purified chondro/osteogenic protein becomes available, then the polyanhydride polymers may prove an even more useful delivery vehicle.

The composition of the present invention can be used in manufacturing controlled-release delivery vehicles, which can be manufactured by recognized methods for preparing controlled-release vehicles. See, U.S. 4,391,727; U.S. 4,767,628. In one embodiment, the present compositions were formed by mixing the selected anhydride polymer and the bioactive factors. Briefly, the polyanhydride polymers or co-polymers were synthesized by

art-recognized techniques. Leong et al., J. Biomed. Mat. Res., 19:941-955 (1985); Conix, Macro. Synth., 2:95-98 (1966). The polymers were ground and sieved into a particle size range of from about 90 to about 150 μm. The water-soluble proteins (i.e., bioactive factors) were mixed with the polymer at the desired ratios, by weight. The relative proportions of polymer to protein will vary depending upon the activity of the protein and the end use of the delivery vehicles. Generally, the protein is present in an amount sufficient, upon release, to interact with the local cell population. The proportion of polymer or co-polymer to protein suitable for the purpose of the present invention will range from about 10% by weight to about 90% by weight of polymer to about 90% to 10% by weight of protein. The preferred amount of protein is from about 20% to about 60% by weight, formulated with sufficient polymer matrix to give 100 parts by weight of the composition.

The mixture of protein and polymer is then molded to form delivery vehicles, which can be shaped in a wide variety of shapes, sizes and forms for delivering the selected bioactive factors to the environemnt of use. For example, the composition can be shaped as buccal and oral devices or articles, vaginal and intrauterine devices or articles, subcutaneous implants or intramuscular devices of cylinderical, bullet, elliptical, circular, disk or other shape that is appropriate for placement in the physiological environments.

Other articles made according to the invention include implants, prostheses or artificial glands for dispensing a water-soluble bioactive factor to a local cell population. In this embodiment, the polymer matrix acts as a support for the surrounding bone, or cartilage tissue.

The composition of the present invention can be formulated for delivering a soluble, bioactive factor to a local cell population to produce the desired localized effect. The composition can be used in animals, including warm-blooded animals, humans, primates, farm and sport animals, laboratory animals, reptiles and amphibians. The amount of bioactive factor is, in one embodiment, the amount necessary to affect the local cell population, or an excess of that amount. Generally, the article can contain from about 5 mg to about 30 mg of protein.

The articles made from the composition of the present invention can be manufactured by standard techniques, such as casting or compression molding. Other methods of shaping polymeric materials into articles having the desired size and shape are well known. In one embodiment of the present invention, the dried polymer is blended with the protein in the desired proportion, and the mixture is pressed into circular disks by compression molding. Compression-molded articles can then be further sectioned into pieces having the desired dimensions.

Polyanhydrides have demonstrated the characteristics necessary for a successful delivery vehicle of osteogenic factors. They released the

inductive protein(s) at an effective dose over a time period coincident with the accumulation of host target cells, as evidenced by the appearance of cartilage and/or bone at the ectopic site. In addition, there was rapid ingrowth of host tissue to promote direct interaction of bioactive factors with the target cells. The polymers are biocompatible, and the composition is biodegradable so that it will ultimately be resorbed.

It should be emphasized that the water-soluble proteins for use in the present invention although capable of inducing a biological effect in vitro do not induce an effect when implanted alone into the physiological site. Similiarly, the polymers themselves do not induce an effect when implanted alone. Only the combination of water-soluble proteins and polymer was effective.

Polyanhydride polymers have now been shown capable of delivering bioactive factors which act on local cell populations. In addition to chondro/osteogenic factors, bioactive factors such as TGF-beta, EGF, FGF, and PDGF which act upon local cell populations in would healing or angiogenesis, can be used in the present devices.

The invention is further illustrated by the following exemplification.

EXEMPLIFICATION

MATERIALS AND METHODS

Preparation of water-soluble proteins from bone matrix

Water-soluble proteins were prepared from a 4 M guanidine hydrochloride extract of demineralized bovine cortical bone as described by Syftestad et al. Syftestad et al., Differentiation, 29:230-237 (1985). Briefly, mid-shaft femoral cortices of 1 year old steers were cleaned of adhering tissue and marrow, decalcified in 0.6 N HCl at 4°C, defatted in chloroform:methanol (1:1 v/v) and air dried. The bone matrix was extracted at 4°C in a solution of 4 M guanidine hydrochloride buffered with 50 mM Tris, pH 6.8, and containing enzyme inhibitors. The extract was dialyzed at 4°C sequentially against solutions of decreasing ionic strength: 0.5 M guanidine hydrochloride, 50 mM Tris buffered saline, and distilled water. Precipitates which formed at each step were removed by centrifugation until only those proteins soluble in cold distilled water remained. This portion of the extract was lyophilized and will hereafter be referred to as water-soluble proteins.

Preparation of polyanhydride polymers

Poly[bis(p-carboxyphenoxy)propane anhydride] (PCPP) and poly[bis(p-carboxy)methane anhydride] (PCPM) were synthesized by melt polycondensation.

Conix, Macro. Synth., 2:95-98 (1966); Leong et al., J. Biomed. Mat. Res., 19:941-955 (1985). Briefly, the dicarboxylic acid monomers were converted to the mixed anhydride by total reflux in acetic anhydride followed by recrystallization. The prepolymers were then subjected to melt polycondensation in vacuo under nitrogen sweep. Copolymers of PCPP and sebacic acid (SA) were obtained in a similar manner. The polymers were purified by extraction with anhydrous ether in a Soxhlet Extractor for several hours and were stored in a desicator over calcium chloride.

Matrices incorporating water-soluble protein were formulated by compression molding. The polymers were ground in a Micro Mill Grinder and sieved into a particle size range of 90-150 μm. Twenty mg of water-soluble proteins were manually mixed with the polymer at the desired ratios (w/w) and the mixture pressed into circular discs in a Carver Test Cylinder Outfit at 30 Kpsi and room temperature. The dimensions of the devices were 14 mm in diameter and 0.9 to 1.1 mm thick. They were manually sectioned into 1 mm^3 disks immediately prior to implantation.

In vivo assay

Under Metophane anaesthesia, a 1 cm incision was made in the dorsal thigh of 5-7 week old CBA/J mice. The implants were placed between muscle beds, care being taken to avoid contact with the femur. The wound was sealed by clips and swabbed with

alcohol. The animals were monitored for signs of inflammation or obvious discomfort, at which time the animal was euthanized and the implant discarded. NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) were observed. The healthy samples were removed 9 or 16 days post-implantation fixed in Perfix (Fisher Scientific), decalcified (if necessary), and processed for histology. Alternate paraffin-embedded sections 5-6 μm thick were stained with either Toluidine blue or Mallory-Heidenhain's stain.

Cartilage was identified by its typical morphology of round cells embedded in an extensive extracellular matrix and by the characteristic metachromatic staining of the extracellular matrix with Toluidine blue. Bone was identified by its characteristic morphology of lining osteoblasts, multinucleated osteoclasts, and osteocytes embedded osteoid and by the dark blue staining of the osteoid with Mallory-Heidenhain's stain. No attempt was made to quantitate the amount of cartilage and/or bone present.

RESULTS

The water-soluble protein preparation from bovine bone matrix used in this study has been previously shown to consist of numerous Coomassie Blue stained protein bands ranging in size from 10 Kd to 100 Kd when subjected to SDS-PAGE. Syftestad et al., Differentiation, 29:230-237 (1985). The water-soluble proteins are capable of inducing

chondrogenesis in two in vitro assay systems: the stage 24 chick limb bud system (Syftestad and Caplan, Dev. Biol., 104:348-356 (1984); Syftestad et al., Differentiation, 29:230-237 (1985)), and day 11 chich embryonic minced muscle explants. Lucas et al., Differentiation, 37:47-52 (1987). When properly formatted in a controlled-release device of the present invention, this protein mixture can also induce ectopic endochondral ossification in the muscle of CBA/J mice. When 20 mg of lyophilized water-soluble proteins were implanted alone into a mouse thigh muscle, however, no signs of cartilage or bone formulation could be detected. Nine days after implantation a "nodule" of connective tissue composed of fibroblastic cell types embedded in a loose extracellular matrix was present. This response is most probably due to the normal wound healing response and was not directly initiated by the implanted water-soluble proteins. By 16 days post-implantation, this connective tissue infiltrate has disappeared making it impossible to locate the original implant site.

Implantation of the polymers, PCPP-SA co-polymer, PCPP, or PCPM, alone without the addition of water-soluble proteins also resulted in the accumulation of connective tissue composed of fibroblastic cells as described above. At 9 days post-implantation, polymer and connective tissue cells were visible for PCPP, and PCPP-SA. The connective tissue was unchanged at 16 days post-implantation. None of the polymer implants alone exhibited any formation of cartilage and/or bone (Table 1).

TABLE 1

Vehicle	Amount of Water-soluble Protein	Number of Implants with Cartilage or Bone/ Number of Implants
Water-soluble proteins	20 mg	0/8
PCPP-SA 30:70	0 mg	0/6
PCPP-SA 30:70		
20% loading	20 mg	0/4
30% loading	20 mg	0/4
40% loading	20 mg	0/4
PCPP	0 mg	0/6
PCPP		
20% loading	20 mg	0/4
40% loading	20 mg	0/4
60% loading	20 mg	3/10
PCPM	0 mg	0/6
PCPM		
20% loading	20 mg	0/4
30% loading	20 mg	6/12
40% loading	20 mg	0/4
50% loading	20 mg	0/4

When water-soluble proteins were incorporated into PCPP-SA copolymers (ratio of PCPP:SA was 30:70) with 20% loading of protein, the result was the accumulation of connective tissue and inflammatory cells 9 days post-implantation. There was no observable cartilage and/or bone in any of the implants (Table 1). Increasing the loading of protein to 30 and 40% did not result in the induction of cartilage and/or bone (Table 1).

PCPP was tested at protein loadings of 20% and 40%. At these loadings, the implants exhibited connective tissue 9 days post-implantation and were essentially indistinguishable from controls. No cartilage and/or bone was induced (Table 1). However, when the loading was increased to 60%, cartilage was observed at 9 days post-implantation and bone at 16 days post-implantation. The induced cartilage and bone was formed adjacent to the pieces of polymer. The incidence of osteogenesis in the implants was 30%.

PCPM was tested at a variety of loading with water-soluble proteins: 20, 30, 40 and 50%. Only the implants loaded with 30% protein exhibited cartilage and/or bone induction (Table 1). The others contained connective tissue and were essentially identical to implants of PCPM alone. However, at 40% loading, cartilage was observed at day 9 post-implantation. The nodules of cartilage were being invaded by vaculature and the beginning of osteogenesis was observable. The nodules of cartilage and bone were usually formed adjacent to

the particles of polymer, but occasionally small particles of polymer could be discerned within the nodule of cartilage. By 16 days post-implantation the cartilage had been replaced by a nodule of trabecular bone. The incidence of induction of cartilage and/or bone in the implants was 50% (Table 1).

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A composition for the controlled release of a bioactive substance, wherein said composition comprises:

a. a shaped matrix sized and adapted for administration of a bioactive substance to an animal and formed of a bioerodible pharmaceutically acceptable material, which material comprises a surface-eroding polymer; and

b. a therapeutically effective amount of a bioactive substance selected from the group consisting of locally acting factors present in the matrix;

wherein the composition bioerodes at a controlled rate over a period of time, thereby administering the bioactive substance to the animal.

2. A composition of Claim 1, wherein the surface-eroding polymer is selected from the group consisting of polyanhydrides and polyorthoesters.

3. A composition of Claim 2, wherein the polyanhydride is selected from the group consisting of:
poly[bis(p-carboxyphenoxy)propane anhydride],
poly[bis(p-carboxy)methane anhydride] and

poly[bis)p-carboxyphenoxy)propane anhydride]-sebacid acid copolymer.

4. A composition of Claim 1, wherein the bioactive substance is a mixture of cold water-soluble proteins.

5. A composition of Claim 4, wherein the cold water-soluble proteins are proteins derived from demineralized bone matrix, and which proteins are chondrogenic or osteogenic.

6. A composition of Claim 5, wherein the chondrogenic or osteogenic proteins comprise a mixture of proteins derived from demineralized bovine femur bone matrix.

7. A composition of Claim 1, wherein the bioactive substance comprises factors which act on local cell populations.

8. A composition of Claim 7, wherein the bioactive substance is selected from the group consisting of TGF-beta, EGF, FGF and PDGF.

9. A composition of Claim 1 wherein the composition comprises a continuous matrix having a bioactive substance interspersed therethrough and wherein the matrix has a geometric shape and size adapted for placement on the animal and insertion in the animal.

10. A composition of Claim 9, wherein the matrix is sized, shaped and adapted for use as an implant.

11. A composition of Claim 10, which is adapted for releasing bioactive substance intramuscularly.

12. A composition of Claim 1, wherein the bioactive substance is released by controlled bioerosion over a prolonged period of time.

13. A composition for the controlled release of a substance for inducing formation of cartilage and bone in an animal, the composition comprising:

a. a shaped matrix sized and adapted for administration of the cartilage and bone inducing substance, said matrix being formed from a polyanhydride polymer selected from the group consisting of PCPP, PCPM and PCPP-SA; and

b. a cartilage and bone inducing amount of a protein preparation derived from de-mineralized bone matrix comprising a mixture of cold water-soluble proteins capable of inducing chondrogenesis and osteogenesis having a range of molecular weight of from about 10 to about 100 Kd;

wherein the composition when implanted intramuscularly bioerodes at a controlled rate over

a period of time, thereby administering said protein preparation over a period of time.

14. A method of selectively delivering a bioactive substance to a specific physical site in an animal comprising implanting in the animal at the selected site a delivery composition of Claim 1.

15. A method of inducing chondrogenesis and osteogenesis in an animal comprising implanting intramuscularly in the animal a delivery device of Claim 13.

16. A method of selectively delivering a bioactive substance to a specific physical site in an animal comprising the steps of:

a. providing a composition comprising:

i. a shaped matrix sized and adapted for administration of the bioactive substance to the site, formed of a surface-eroding polymer; and

ii. a therapeutically effective amount of a bioactive substance selected from the group consisting of locally-acting factors, present in the matrix; and

b. implanting the composition at the specific physical site in the animal.

17. A method of Claim 16, wherein the surface-eroding polymer is selected from the group consisting of polyanhydrides and polyorthoesters.

18. A method of Claim 17, wherein the polyanhydride is selected from the group consisting of: poly[bis(p-carboxyphenoxy)propane anhydride], poly[bis(p-carboxy)methane anhydride] and poly[bis(p-carboxyphenoxy)propane anhydride]-sebacic acid copolymer.

19. A method of Claim 16, wherein the bioactive substance comprises a mixture of water-soluble proteins derived from demineralized bone matrix, which proteins are chondrogenic or osteogenic.

20. A method of Claim 19, wherein the composition is implanted intramuscularly.

21. A method of Claim 19, wherein the composition is implanted subcutaneously.

22. A method of Claim 16, wherein the bioactive substance is selected from the group consisting of TGF-beta, EGF, FGF and PDGF.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/00900

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^5: A 61 K 9/20, A 61 K 9/22, A 61 K 9/26

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC^5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
Y	WO, A, 89/00855 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 9 February 1989 see page 1, line 1 - page 4, last line; page 12, lines 14-20; page 25, line 1 - page 27, last line --	1-13
Y	Proc. Natl. Acad. Sci. USA, vol. 84, October 1987, (Washington, D.C. US), T.K. Sampath et al.: "Isolation of osteogenin, an extracellular matrix-associated, bone-inductive protein, by heparin affinity chromatography", pages 7109-7113, see the whole article (cited in the application) --	1-13
	./.	

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th May 1990	02.07.90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	F.W. HECK

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Proc. Natl. Acad. Sci. USA, vol. 85, December 1988, (Washington, D.C., US), E.A. Wang et al.: "Purification and characterization of other distinct bone-inducing factors", pages 9484-9488, see the whole article (cited in the application)	1-13

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers *)..., because they relate to subject matter not required to be searched by this Authority, namely:

*) Claims 14-22. See PCT Rule 39.1(iv):

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9000900 SA 34775

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 26/06/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8900855	09-02-89	US-A- 4857311	15-08-89
		AU-A- 2258088	01-03-89
		EP-A- 0368912	23-05-90